

HLA-A Disparities Illustrate Challenges for Ranking the Impact of HLA Mismatches on Bone Marrow Transplant Outcomes in the United States

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HLA disparity between hematopoietic stem cell donors and recipients is one of the most important factors influencing transplant outcomes, but there are no well-accepted guidelines to aid in selecting the optimal donor among several HLA mismatched donors. In this report, HLA-A is used as a model to illustrate factors that are barriers to delineating the relationship between specific HLA mismatches and transplant outcomes in the United States. Patients in this investigation received transplants for hematologic malignancies that were facilitated by the National Marrow Donor Program (NMDP) between 1990 and 2002 (n = 4226). High-resolution HLA typing was performed for HLA-A, -B, -C, -DRB1, -DQA1, -DQB1, -DPA1, and -DPB1. HLA-A mismatches were observed in 745 donor-recipient pairs and 62% of these pairs also had disparities at HLA-B, -C, and/or -DRB1. The HLA-A mismatches involved 190 different combinations of HLA-A alleles and 51% of these were observed in only 1 pair. Addition of a single HLA-A disparity when HLA-B, -C, and -DRB1 were matched (n = 282) was associated with increased mortality (odds ratio [OR] = 1.32, confidence interval [CI] 1.07-1.63). When HLA-B, -C, and -DRB1 were matched, the most frequent HLA-A mismatches were HLA-A*0201:0205 (n = 28), HLA-A*0301:0302 (n = 15), HLA-A*0201:0206 (n = 15), HLA-A*0201:6801 (n = 12), HLA-A*0101:1101 (n = 11), and HLA-A*0101:0201 (n = 10). There were no statistically significant relationships between any of these disparities and transplant outcomes (engraftment, acute and chronic graft-versus-host disease [aGVHD, cGVHD] relapse, treatment-related mortality [TRM], or overall survival [OS]) when adjustments for multiple comparisons were considered. Achieving 80% power to detect an effect of any 1 of these 6 HLA-A disparities on survival is estimated to require a total transplant population of 11,000 to more than 1 million U.S. donor-recipient pairs depending upon the HLA disparity. Thus, alternative approaches are required to develop a clinically relevant ranking system for specific HLA disparities in the United States.

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INTRODUCTION

For bone marrow transplantation (BMT), one of the few factors that the physician can modify to improve transplant outcomes is donor compatibility [1]. An HLA identical donor is optimal, but matched donors are not available for most patients. It is often necessary to prioritize several candidate donors who have different HLA disparities. For example, it may be necessary to choose between (1) a mismatch at the HLA-A or HLA-DRB1 locus, (2) an allele-level mismatch and an antigen-level mismatch, or (3) an allele with 1 amino acid difference and another with 3 amino acid differences. There is limited and sometimes contradictory evidence to support these decisions. Many factors including numerous confounding clinical variables, limitations in the ability to adjust for the effects of multiple

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HLA disparities, and statistical limitations imposed by the size of data sets are barriers to solving this problem [2,3].

For studies comparing different HLA loci, HLA-A, -B, -C, and -DRB1 disparities have most consistently been associated with adverse outcomes [1,4-8]. In 1 of the largest of these studies, there was a 9% to 10% decrement in survival for each HLA-A, -B, -C, or -DRB1 mismatch [1]. Several studies have suggested differences between allele-level and antigen-level disparities [4,8], but 1 of the largest studies using a rigorous statistical model did not confirm this relationship for a single allele versus a single antigen level mismatch [1]. It has been more difficult to detect associations between HLA-DQ and -DP disparities and transplant outcomes, but associations have been reported under certain conditions [1,6,9-11].

Some recent clinical investigations have attempted to investigate particular HLA mismatches or specific amino acid differences. A Japanese study reported 15 combinations of mismatched alleles and 6 specific amino acid differences that were associated with graft-versus-host disease (GVHD) [12]. Because large numbers of comparisons were required to detect this small number of statistically significant combinations and there were many confounding variables, independent confirmation or other supporting data are essential [13]. An international study focused on disparities involving HLA-A*02 alleles reported that mismatches between HLA-A*0201 and HLA-A*0206 were deleterious, but mismatches between HLA-A*0201 and HLA-A*0205 were tolerated [14]. Interestingly, HLA-A*0201 and HLA-A*0206 are distinguished by a single amino acid difference at position 9. HLA-A*0201 and HLA-A*0205 have the same amino acid difference along with 2 additional mismatched amino acids. The HLA-A*0206 mismatches were predominantly observed in Japanese patients, whereas the HLA-A*0205 mismatches were in non-Japanese patients.

Fundamental knowledge about the molecular mechanisms underlying allorecognition should be useful in prioritizing HLA mismatches. There is a large body of research using animal models as well as in vitro assays that show that the molecular characteristics of Major Histocompatibility Complex (MHC) disparities influence allorecognition. Alloreactive T lymphocytes play a key role in graft rejection, GVHD, and graft-versus leukemia (GVL) effects. These T cells respond to differences between HLA molecules and/or peptide antigens bound by HLA molecules [15,16]. Elsner et al. [17] used these concepts to rank HLA disparities based upon the number of amino acid differences between HLA molecules with adjustments for amino acid position and a similarity index (Risler score). However, in vitro and clinical observations have not supported this particular approach [18,19]. Another algorithm, HLA Matchmaker, which uses amino acid

triplets within the HLA molecule, showed some promise in solid organ transplantation, but was not useful for BMT [20].

The long-term goal of this investigation is to utilize a combination of fundamental knowledge about allorecognition along with clinical outcomes to develop a new system for evaluating candidate donors who are HLA mismatched. The purpose of the study described here is to delineate the HLA factors that need to be considered to investigate the impact of HLA disparities and to test the hypothesis that there are certain common HLA disparities that are deleterious. HLA-A was selected as the model for testing this hypothesis because HLA-A mismatches have been associated with adverse outcomes in every large clinical investigation, and a recent NMDP report suggests that mismatches at HLA-A or -DRB1 may have greater effects on survival than HLA-B or -C mismatches [1]. The data reported here show the limitations posed by the diversity of HLA disparities in unrelated donor transplants performed in the United States and present transplant outcomes for the most frequent HLA-A disparities.

MATERIALS AND METHODS

Patients

This investigation was approved by the National Marrow donor Program (NMDP) institutional review board. Adults transplanted under the auspices of the NMDP between 1990 and 2002 for acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), or non-Hodgkin lymphoma (NHL) who had high resolution typing for HLA-A, -B, -C, -DRB1, -DQA1, -DQB1, -DPA1, and -DPB1 were eligible for this investigation. The subject population was adjusted as described previously to correct for potential bias that may have been created by consent practices [1]. Early-stage disease was defined as AML and ALL in first complete remission (CR), CML in first chronic phase, and MDS subtype refractory anemia (RA). Intermediate-stage disease was AML or ALL in second or subsequent CR or in first relapse, and CML in accelerated phase or second chronic phase. Advanced-phase disease was AML in second or higher relapse or primary induction failure, CML in blast phase, MDS subtypes refractory anemia with excess blasts (RAEB) or in transformation, or MDS, not otherwise classified. NHL cases were not classified for disease stage.

Characteristics of the patients (n = 4226) and donors are provided in Supplemental Table 1. Demographic data were submitted by the transplant centers and subjected to a rigorous quality assurance process including on-site auditing against medical records, double data entry, on-line edit checks, and review of the data for

inconsistencies and omissions. Race was self-reported. The majority of the recipients and donors were Caucasian (recipients: 87% and donors: 85%). For cases where race was reported for both donor and recipient, 87% of the pairs reported the same race/ethnicity. The majority of patients received myeloablative (MA) conditioning regimens (93%) and calcineurin inhibitor-based GVHD prophylaxis (78%).

HLA Matching

Allele-level HLA typing was performed for HLA-A, -B, -C, -DRB1, -DQ, and -DP using methods that were previously described in detail [21]. Briefly, DNA-based HLA typing was based on exons 2 and 3 for HLA Class I and exon 2 for HLA Class II. Known null alleles were excluded using serologic typing or extended DNA-based typing.

HLA mismatching was determined by comparing the peptide sequences encoded by the exons that were used for HLA typing (ie, exons 2 and 3 for HLA Class I and exon 2 for HLA Class II) using a sequence database for the human major histocompatibility complex (MHC) [22]. Low-resolution HLA mismatches were defined as differences in the first 2 digits of the HLA types (eg, HLA-A*0101 versus HLA-A*0201), and high-resolution mismatches were defined as differences in only the last 2 of 4 digit HLA types (eg, HLA-A*0201 versus HLA-A*0205). Particular combinations of mismatched alleles in this study are reported as HLA locus*first allele:second allele. For example, a mismatch involving HLA-A*0101 and HLA-A*0201 is denoted A*0101:0201. HLA-DQ and -DP disparities were not included in the assessment of overall HLA matching because mismatches at these loci did not individually have detectable effects on survival. If a donor had a homozygous HLA type and the recipient was heterozygous at the same locus, the mismatch was treated as a unidirectional mismatch for GVHD. Conversely, mismatches were treated as unidirectional for engraftment if a recipient had a homozygous HLA type and the donor was heterozygous.

For analysis of HLA-A mismatches, the orientation of amino acid side chains was based upon the position of the side chain in HLA-A crystal structures [23-25] as well as peptide contact residues defined by Chelvanayagam [26]. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco.

Clinical Outcomes

The primary endpoint for this analysis was overall survival (OS), which was defined as the time from graft infusion (day 0) to death from any cause. Several secondary endpoints were also examined. Engraftment

was defined as maintaining an absolute neutrophil count (ANC) $>500 \times 10^6/L$ for 3 consecutive measurements by day 28. Acute GVHD (aGVHD) was graded using the Glucksberg scale [27], and chronic GVHD (cGVHD) was graded using the method of Shulman et al. [28]. Disease relapse was defined using criteria determined by the Center for International Blood and Marrow Transplant Research (CIBMTR). Treatment-related mortality (TRM) was defined as death while in continuous CR from the primary disease.

Statistics

Continuous variables are presented as the medians and ranges. Categorical variables are reported as percentages. The Kaplan-Meier estimator was used for determining probabilities for survival endpoints with variance estimated by Greenwood's formula. Comparisons of survival curves were done with the log-rank test. Multivariate analyses were performed using the proportional hazards model to compare the HLA matched and mismatched groups. Models were fit to determine which risk factors may be related to a given outcome. All models were tested for significant clinical covariates including disease, disease stage, Karnofsky performance status, donor-patient cytomegalovirus (CMV) serology, patient race, patient age, T cell depletion, use of total body irradiation (TBI), graft source (peripheral blood (PB) or BM), donor age, patient-donor sex match, and year of transplant. Models included any clinical factors that were related to a given outcome at $P < .05$. All variables were tested for the affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption were adjusted first before the stepwise model building approach was used in developing models for the primary and secondary outcomes. Because of multiple comparisons, a threshold of $P < .01$ for the main effect was used for significance.

RESULTS

Characteristics of HLA-A Disparities

HLA-A mismatches were observed in 745 donor-recipient pairs, and 62% of these pairs also had disparities at HLA-B, -C, and/or -DRB1. One of the factors that many transplant centers use to classify HLA disparities is the level of typing resolution required to detect the mismatch: high-resolution types (allele-level) or low-resolution types (antigen level). Figure 1 shows that these classifications are generally indicative of the number of amino acid differences between 2 HLA-A molecules with low-resolution mismatches having 3-28 amino acid differences and high-resolution mismatches having 1-6 amino acid differences. For low-resolution disparities, there are a mean 13.5 amino acid differences

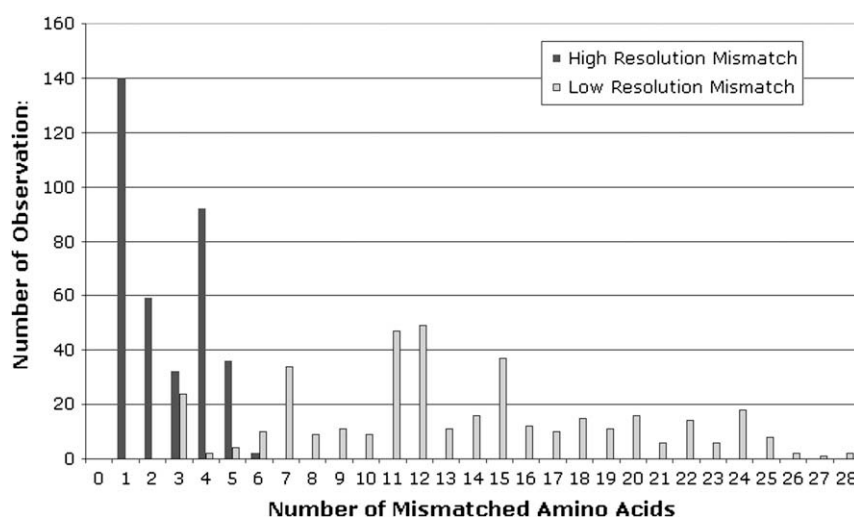


Figure 1. Number of mismatched amino acid residues in each HLA-A disparity in 4226 donor-recipient pairs.

(median 12) and most of the disparities have more than 6 amino acid differences. In this study there are 4 “low-resolution” mismatch combinations that have only 3 amino acid differences: A*0101:3601 ($n = 2$), A*2301:2402 ($n = 11$), A*2601:6601 ($n = 8$), and A*3101:3303 ($n = 3$). The HLA-A mismatches with the largest number of amino acid differences ($n = 28$) are A*2301:3601 ($n = 1$) and A*2301:6601 ($n = 1$).

High-resolution HLA-A mismatches have only 1–6 mismatched amino acids (mean 2, median 2). Six amino acid differences are observed in only 1 relatively uncommon disparity, A*3001:3004 ($n = 2$). In this dataset there are 26 different HLA-A high resolution mismatches that have a single amino acid difference. These single amino acid differences are located at 19 different positions within the HLA-A molecule (3, 9, 19, 36, 54, 66, 70, 74, 87, 97, 99, 102, 116, 127, 131, 144, 156, 163, and 171). Ten of these are predicted to have side chains that contact the peptide bound to the HLA molecule [26]. These data show that HLA disparities defined by a single amino acid difference constitute a diverse group, because the functional role of the mismatched amino acid is extremely variable.

There has been interest in determining if HLA mismatches involving particular amino acid positions are associated with unfavorable outcomes. In general, the amino acid positions that are observed in the largest number of different mismatch combinations (Table 1) influence the peptide binding groove of the HLA molecule (Figure 2). However, there are also 54 different amino acid positions that are involved in only 1 mismatch combination. These are distributed throughout the HLA molecule and each of these combinations is infrequent (1–5 observations/combination). Because the impact of a particular amino acid position is influenced by the molecular context of the amino acid disparity, position alone is unlikely to be sufficient for predicting effects. Position 9 exemplifies the potential

for numerous interactions that are likely to confound comparisons that are focused on particular positions within the HLA molecule. In this study, position 9 is mismatched in 389 of the HLA-A disparities and these disparities involve 105 different combinations of HLA-A molecules. Three of these combinations involve a single amino acid mismatch ($n = 64$) with the majority being A*0201:0206 ($n = 60$). The remaining mismatch combinations that involve a difference in position 9 have up to 27 additional amino acid differences. The most frequent numbers of additional amino acid differences are 4 ($n = 67$, 3 combinations), 12 ($n = 47$, 7 combinations), and 11 ($n = 41$, 9 combinations). Considering this extensive variability, a study grouping any amino acid difference at position 9 is questionable from the functional perspective.

Many of the highly variable amino acid positions are predicted to influence the peptides that can bind to the HLA molecule and/or their precise position within the peptide binding groove. This can be examined using 9 peptide environments that have been shown to alter the amino acid

Table 1. Amino Acids that Are Different in the Largest Number of HLA-A Mismatch Combinations

Amino Acid Position*	HLA-A Mismatch Combinations (n)
9	105
62	99
63	72
70	74
76	84
77	76
97	91
114	97
116	71
156	90
152	85

*Based upon numbering of amino acids in the mature protein.

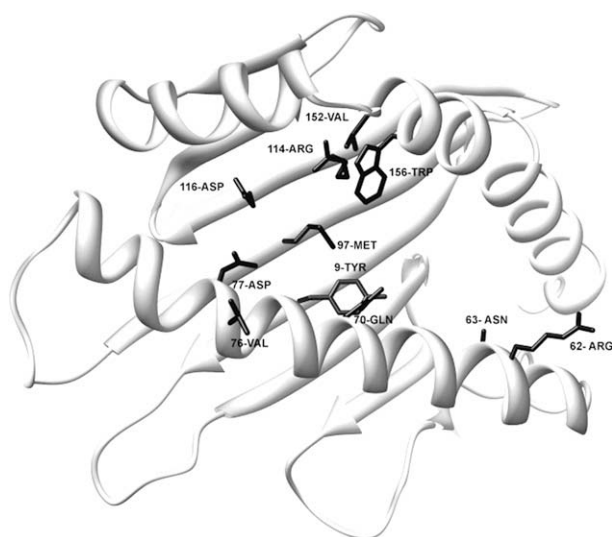


Figure 2. Structure of HLA*6801. Amino acids that are observed in the largest number of different mismatch combinations are distributed throughout the peptide binding domain. The side chains of the amino acids that are observed in the largest number of different mismatch combinations are shown on an HLA-A*6801 structure (2HLA in the Protein Data Bank). Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco.

preference for each of the positions in a nonamer peptide [26]. In the donor-recipient pairs in this study, more than half of the 54 amino acid positions that were disparate influence these peptide binding environments. If the frequency of each mismatch combination is considered, the amino acids that are most often mismatched are all located in positions that alter these 9 peptide binding environments: positions 9 ($n = 389$), 76 ($n = 255$), 97 ($n = 251$), 114 ($n = 251$), 156 ($n = 362$), 152 ($n = 248$), 77 ($n = 241$), 95 ($n = 239$), 62 ($n = 229$), 70 ($n = 225$), and 116 ($n = 204$), where n is the number of donor-recipient pairs with a mismatch at the position. With the exception of position 95, these frequently mismatched amino acids influence several of the 9 peptide binding environments. The effects on peptide binding are expected to be extremely variable.

Multiple HLA Disparities

The clinical impact of a particular HLA-A disparity may be confounded by the effects of additional mismatches at other HLA loci. The high frequency of multiple HLA locus mismatches is demonstrated in Figure 3, which shows each amino acid position that was mismatched at HLA-A in this subject population along with the proportion of mismatches that involve additional mismatches at HLA-B, -C, and -DRB1. Overall, 62% of the HLA-A mismatches are accompanied by additional disparities at HLA-B, -C, and/or -DRB1.

Most Frequent HLA-A Disparities

Table 2 lists the 16 HLA-A disparities that were observed at least 10 times in the entire data set (including those with additional mismatches at HLA-B, -C, and/or DRB1). Investigating the effects of particular HLA mismatches or mismatched positions is simplified by focusing on single HLA mismatches, because strategies to compensate for additional disparities are currently suboptimal. If HLA-A disparities are considered only when HLA-B, -C, and -DRB1 are matched, there are only 6 mismatch combinations that are observed at least 10 times in a population of 4226 transplants. These HLA-A mismatch combinations have very different properties including differences in the number of mismatched amino acids (1-24 amino acids, Table 4). One common feature is that all of them have at least 1 amino acid difference located in a position that is predicted to influence the peptides bound to the HLA molecules.

Clinical Outcomes for the Most Frequent HLA-A Disparities

Because knowledge regarding the interactions caused by multiple HLA disparities is extremely limited, outcomes analysis was limited to pairs having a single HLA-A mismatch with matching at HLA-B, -C, and -DRB1. To select the reference population, donor-recipient pairs with 8/8 matches (HLA-A, -B, -C, and -DRB1), 12/12 matches (HLA-A, -B, -C, -DRB1, -DQA1, and -DQB1), and 16/16 matches (HLA-A, -B, -C, -DRB1, -DQA1, -DQB1, -DPA1, and -DPB1) were compared. For OS, there were no differences between the 16/16 matched group and those with HLA-DP mismatches (12/12 matches, odds ratio [OR] = 1.00, 95% confidence interval [CI] 0.84-1.18) or those with HLA-DP and/or -DQ mismatches (8/8 matches, OR = 1.01, 95% CI 0.80-1.29). Therefore, the 8/8 matched group was used as the reference group for pairs with HLA-A mismatches. A single disparity at the HLA-A locus was associated with increased mortality (OR = 1.32, 95% CI 1.07-1.63).

To determine if it is possible to detect detrimental effects of any of the most common HLA-A mismatches, transplant outcomes were examined for HLA-A mismatches that were observed in at least 10 pairs when HLA-B, -C, and -DRB1 were matched. There are only 6 HLA-A mismatches that met these criteria: A*0201:0205, A*0301:0302, A*0201:0206, A*0201:6801, A*0101:1101, and A*0101:0201 (Table 3). The patient and donor characteristics of these pairs that are provided in Table 3 show that there were no major differences among these groups. For OS, with relatively small numbers of patients there are no statistically significant differences between pairs with these HLA disparities and the 8/8 matched pairs (data not shown). The most common

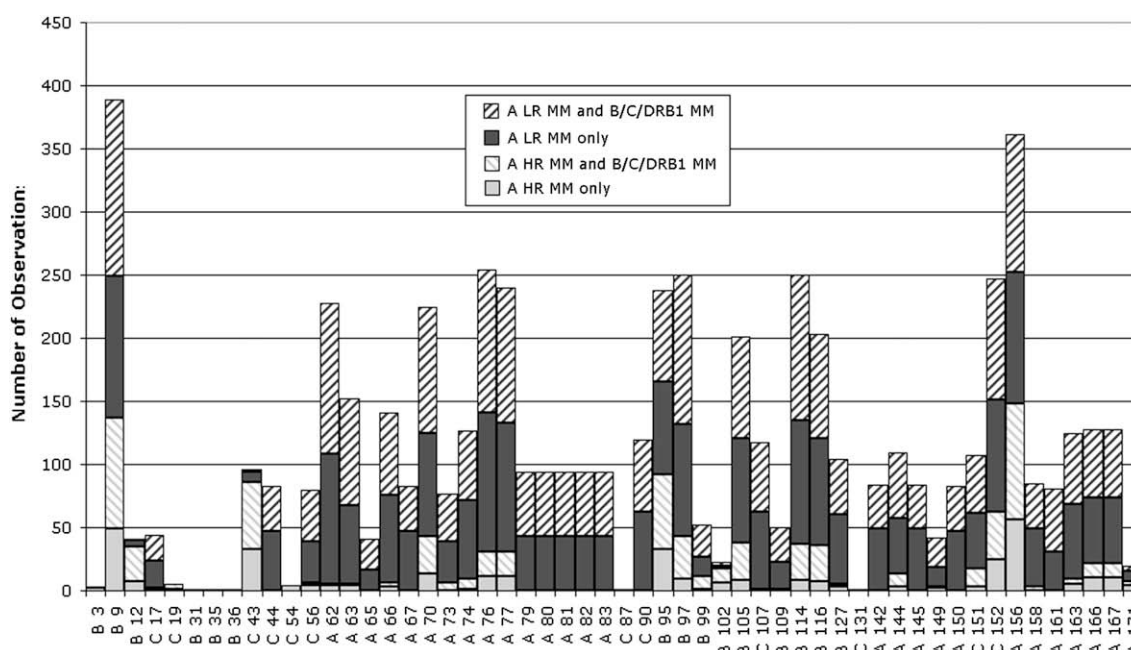


Figure 3. Amino acid differences that are involved in HLA-A disparities are distributed throughout the peptide binding domain, present in both low-resolution (LR) and high-resolution (HR) mismatches, and often accompanied by mismatches (MM) at HLA-B, -C, and -DRB1 loci. The position and side-chain orientation of each mismatched amino acid are indicated below the x-axis (A = alpha helix, B = beta sheet, and C = connecting loop). The amino acids that are most frequently mismatched line the peptide binding groove of the HLA molecule. Most of the mismatched amino acids are involved in both low- and high-resolution mismatches.

single HLA-A mismatch is A*0201:0205 (OR = 2.08, 95% CI 0.86-4.80, $P = .07$), but this did not reach statistical significance. Multivariate results for survival of pairs with the 6 HLA mismatches compared with the 8/8 matched pairs has an overall P -value of .075.

For severe aGVHD (grades III-IV), the overall P -value for comparison of these mismatches with 8/8 matches is .014, and data for the individual HLA-A mismatches are shown in Table 4. Interestingly,

A*0201:0206, which was found to have an association with aGVHD in another study [12], is approaching significance in this study (OR = 3.22, 95% CI 1.02-10.50, $P = .03$). There were no significant differences detected for engraftment (overall P -value .36), cGVHD (overall P -value = .28), relapse (overall P -value = .19), TRM (overall P -value = .14), or disease-free survival (DFS) (overall P -value = .18) (data not shown).

Table 2. HLA-A Disparities Observed in at Least 10 Donor-Recipient Pairs

HLA MM	Number of Observations in 4226 Pairs	Number of Observations in HLA-B, -C, -DRB1 Matched Pairs (%)	Number of Mismatched Amino Acids
0201-0205	65	28 (43)	4
0201-0206	60	15 (25)	1
6801-6802	34	7 (21)	5
0201-6801	30	12 (40)	12
0301-0302	27	15 (56)	2
3001-3002	23	7 (30)	4
0101-1101	23	11 (48)	11
0301-1101	19	8 (42)	7
0101-0301	19	9 (47)	15
2901-2902	17	6 (35)	1
2402-2403	17	9 (53)	2
0201-0202	16	4 (25)	3
0101-0201	13	10 (77)	24
2501-2601	12	7 (58)	7
3101-3201	12	5 (42)	12
2301-2402	11	3 (27)	3

MM indicates mismatched.

DISCUSSION

One of the major challenges in BMT is selection of the most compatible donor when an HLA matched donor is not available. Although several investigations have attempted to determine which HLA disparities are poorly tolerated, the results have often been contradictory. The investigation reported here uses one of the largest study populations to date ($n = 4226$) to illustrate that extensive HLA diversity is a major barrier to establishing the association between HLA disparity and transplant outcomes.

HLA-A was selected for this investigation because mismatches at this locus have been most consistently associated with adverse transplant outcomes [1,4,5,12]. In the 4226 pairs examined in this investigation, there were 190 different HLA-A mismatch combinations and 51% of these were observed in only 1 pair. Very few HLA-A disparities were observed frequently enough to study.

Table 3. Demographics of Patients and Donors for the 6 Most Frequent HLA-A Disparities in HLA-B, -C, and -DRB1 Matched Pairs

Variable	A*0201-A*0205 N (%)	A*0301-A*0302 N (%)	A*0201-A*0206 N (%)	A*0201-A*6801 N (%)	A*0101-A*1101 N (%)	A*0101-A*0201 N (%)
Patients (n)	28	15	15	12	11	10
Centers (n)	22	12	13	9	7	8
Patient Age						
Median (years)	27	37	23	32	42	29
Range (years)	1-51	9-58	3-51	1-54	2-50	13-45
<20 years	7 (25)	1 (6)	7 (46)	4 (34)	2 (18)	4 (40)
20-39 years	14 (50)	7 (47)	4 (27)	4 (33)	3 (27)	5 (50)
40+ years	7 (25)	7 (47)	4 (27)	4 (33)	6 (55)	1 (10)
Male sex	14 (50)	7 (47)	10 (67)	7 (58)	4 (36)	7 (70)
Karnofsky \geq 90	21 (78)	12 (80)	12 (80)	8 (73)	7 (64)	9 (90)
Disease						
AML	7 (25)	1 (7)	5 (33)	5 (42)	3 (27)	4 (40)
ALL	10 (36)	1 (7)	3 (20)	1 (8)	0	0
CML	6 (21)	11 (73)	7 (47)	3 (25)	6 (55)	4 (40)
MDS	4 (14)	2 (13)	0	2 (17)	2 (18)	2 (20)
NHL	1 (4)	0	0	1 (8)	0	0
Disease status						
Early	7 (25)	9 (60)	6 (40)	4 (33)	4 (36)	6 (60)
Intermediate	11 (39)	4 (27)	9 (60)	5 (42)	4 (36)	2 (20)
Advanced	8 (29)	2 (13)	0	1 (8)	1 (9)	1 (10)
Other	2 (7)	0	0	2 (17)	2 (18)	1 (10)
Marrow	28 (100)	15 (100)	15 (100)	11 (92)	10 (91)	9 (90)
Conditioning						
Ablative	27 (96)	14 (93)	15 (100)	12 (100)	11 (100)	10 (100)
Other	1 (4)	1 (7)	0	0	0	0
GVHD Prophylaxis						
FK506	5 (18)	2 (13)	2 (13)	4 (33)	0	1 (10)
CsA	17 (61)	10 (67)	13 (87)	6 (51)	10 (91)	6 (60)
T cell depletion	6 (21)	3 (20)	0	1 (8)	1 (9)	3 (30)
Other	0	0	0	1 (8)	0	0
Donor/recipient sex match						
M/M	10 (36)	6 (40)	7 (47)	4 (33)	2 (18)	3 (30)
M/F	10 (36)	3 (20)	3 (20)	2 (17)	5 (46)	2 (20)
F/M	4 (14)	1 (7)	3 (20)	3 (25)	2 (18)	4 (40)
F/F	4 (14)	5 (33)	2 (13)	3 (25)	2 (18)	1 (10)
Donor/recipient CMV match						
N/N	14 (50)	7 (47)	2 (14)	3 (25)	5 (46)	4 (40)
N/P	6 (22)	6 (40)	3 (20)	2 (17)	3 (27)	2 (20)
P/N	4 (14)	0	5 (33)	1 (8)	2 (18)	3 (30)
P/P	4 (14)	2 (13)	5 (33)	5 (42)	1 (9)	0
Unknown	0	0	0	1 (8)	0	1 (10)
Donor age (years) Median (range)	40 (20-57)	39 (21-50)	36 (20-53)	36 (29-47)	39 (22-53)	38 (22-49)
Year of transplant						
1990-1994	6 (21)	3 (20)	3 (20)	1 (8)	3 (27)	5 (50)
1995-2002	22 (79)	12 (80)	12 (80)	11 (92)	8 (73)	5 (50)
Follow-up median months (range)	60 (48-101)	111 (36-144)	61 (22-99)	77 (35-97)	90 (60-157)	55 (34-154)

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; GVHD, graft-versus-host disease; CsA, cyclosporine; N, number; M, male; F, female; CMV, cytomegalovirus.

Only 4 HLA-A disparities were observed in 30 or more donor-recipient pairs (A*0201:0205, A*0201:0206, A*6801:6802, A*0201:6801). Sixteen and HLA disparities were observed in 10 or more donor-recipient pairs (Table 2). If pairs with additional HLA-B, -C, and -DRB1 disparity are excluded to diminish confounding HLA variables, only 6 mismatch combinations occur in 10 or more pairs (Table 4). Because the most frequent HLA-A mismatches in HLA-B, -C, and -DRB1 matched pairs occur only 2-6 times/1000 U.S. patients, it is not feasible to directly determine associations between a particular HLA-A disparity and transplant outcomes for patients transplanted in the United States.

One of the goals of this investigation was to identify frequent HLA disparities that are deleterious. However, there were only 6 mismatch combinations observed more than 10 times in HLA-B, -C, and -DRB1 matched pairs and when survival of each of these small groups was compared with that of the HLA-matched control group, no statistically significant associations were detected. A*0201:0205, the most frequent HLA-A disparity observed when HLA-B, -C, and -DRB1 were matched, had a trend toward increased mortality at 1 year ($P = .07$, OR = 2.08, 95% CI 0.86-4.80). A study from the 14th International Histocompatibility Workshop examined 51 pairs with A*0201:0205 mismatches using additional

Table 4. Transplant Outcomes for the 6 Most Frequent HLA-A Disparities in HLA-B, -C, and -DRB1 Matched Pairs

HLA Mismatch	A*0201:0205	A*0301:0302	A*0201:0206	A*0201:6801	A*0101:1101	A*0101:0201
Number of amino acid differences	4	2	1	12	11	24
Position and single-letter code for amino acid differences	9FY 43QR 95VL 156LVW	152EV 156LQ	9FY	9FY 62GR 63EN 66KN 70HQ 74HD 95VI 97RM 107WG 114HR 116YD 156LW	9FY 44KR 67MV 70HQ 76AV 77ND 150VA 156RQ 158VA 166DE 167GW	44KR 62QG 66NK 67MV 74DH 76AV 77ND 90DA 95IV 97IR 107GW 114RH 116DY 127NK 142IT 145RH 150VA 152AV 156RL 158VA 163RT 105PS 166DE 167GW
N	27*	15	15	11*	11	9*
OR for survival	2.08	1.10	2.64	2.52	1.13	0.86
95% CI	0.86-4.80 ($P = .07$)	0.25-3.73	0.81-8.38	0.60-9.94	0.19-4.74	0.09-4.55
OR for acute GVHD	1.41	1.03	3.22	1.06	2.35	0.0
95% CI	0.55-3.33	0.24-3.48	1.02-10.50 ($P = .03$)	0.18-4.43	0.56-9.29	0.0-1.12

OR indicates odds ratio; CI, confidence interval; GVHD, graft-versus-host disease.

*Three cases (A*0201:0205, A*0201:6801, and A*0101:0201) were not included in the multivariate analysis because of missing data.

HLA mismatches as a covariable and was unable to detect any association between this disparity and survival [14]. Additional donor recipient pairs are required to evaluate the impact of this disparity.

A trend for increased severe aGVHD was detected for one of the most frequent mismatches, A*0201:0206 (OR = 3.22, 95% CI 1.02-10.50, $P = .03$). This is provocative because 2 recent publications reported associations between A*0201:0206 and adverse outcomes. Kawase et al. [12], who studied transplants facilitated by the Japan Marrow Donor Program (JMDP), reported that a graft from an A*0206 donor to an A*0201 recipient was significantly associated with GVHD (OR = 1.78, $P < .001$). An analysis of data from the 14th International Histocompatibility Workshop suggested that A*0201:0206 mismatches were associated with mortality. One limitation in these 2 studies is that it is likely that there is some overlap in the subjects because many of the A*0201:0206 mismatched pairs examined in the Workshop were obtained from the JMDP. Nevertheless, the A*0201:0206 mismatch combination has now been identified as a potential risk factor in several studies, suggesting that this particular mismatch may be associated with a GVHD and perhaps mortality.

A high incidence of certain HLA-A disparities in the JMDP data set allowed Kawase et al. [12] to examine directional differences in HLA disparities. For example, in their analysis an A*0201 donor:A*0206

recipient was not detrimental (OR = 1.23, $P = .22$), whereas A*0201 recipient:A*0206 donor (OR = 1.78, $P < .001$) was significantly associated with GVHD. In their study, there were 3 HLA-A disparities that were associated with aGVHD, and all of these associations were directional. The possibility of directional effects has also been suggested by laboratory studies involving B*4402:4403 where (1) in vitro assays, (2) characteristics of the peptides bound by the 2 HLA molecules, and (3) crystal structures of the molecules have shown that the same peptide in the HLA binding groove can have different orientations in HLA-B*4402 and *4403. These differences can be recognized by T lymphocytes [15,16,29,30], and may be clinically important [31,32]. For studies involving heterogeneous patient populations such as those from the NMDP, there are too few subjects with particular HLA disparities to further subdivide the mismatches according to their presence in the donor or recipient and this limitation may obscure significant effects.

One of the striking differences between reports from the JMDP and NMDP data sets is the incidence of particular HLA disparities. A study of 5210 subjects whose transplants were facilitated by the JMDP reported very large frequencies for certain HLA-A mismatch combinations: A*0201:0206 ($n = 269$), A*2402:2420 ($n = 90$), and A*2601:2603 ($n = 69$). The allelic lineages HLA-A2, A26, and A24 present

rich diversity in the Japanese population [33]. In contrast, the European populations present only a limited number of alleles in these groups because there is a predominant allele in each lineage. The NMDP data set is largely composed of patients and donors with European ancestry where most of the pairs are HLA matched for common alleles and the mismatches often involve low-frequency alleles.

Using the frequencies of the 6 most common HLA-A disparities observed in this study when HLA-B, -C, and -DRB1 are matched and the 100 day mortality difference observed between each disparity and an 8/8 match, the number of U.S. donor recipient pairs needed to achieve 80% power to detect an association with survival ranges from 11,000 to more than 1.3 million, depending upon the HLA mismatch (Table 5). For example, it is estimated that 72 pairs with a single A*0201:0205 would be required to achieve 80% power to detect an association with survival versus an 8/8 match. Using the frequency of 27 pairs with this HLA disparity in 4226 subjects, it is estimated that 11,269 pairs would be required to detect an association with survival. Attempts to overcome this limitation by including pairs with multiple HLA mismatches creates another problem—adjusting for the effects of particular HLA disparities which are not yet well understood.

One alternative that has been explored is to investigate the impact of mismatching particular amino acids within the HLA molecule. Kawase et al. [12] used bootstrap analysis to examine each amino acid involved in an HLA mismatch and concluded that 2 specific amino acid substitutions in HLA-A were associated with aGVHD: position 9 Tyr versus Phe and position 116 Asn versus Asp [12]. This approach, however, has some limitations, and the conclusions about some specific residues may be biased because context of the amino acid differences is ignored. For example, in the NMDP data set residue 9 can be mismatched along with 0-28 additional amino acids; a large body of laboratory investigation suggests that context of an amino acid difference is crucial for

allo-recognition. The analysis conducted by Kawase et al. [12] may also be confounded by the occurrence of additional HLA mismatches in each donor-recipient pair. In this scenario specific amino acid mismatches may occur in 1 or a few mismatch combinations that may be accompanied by an additional mismatch in another locus as the result of linkage disequilibrium. Therefore, the effect attributed to a particular amino acid mismatch at a given locus may, in fact, result from the occurrence of multiple mismatches. Another concern is that multiple comparisons were made in the study by Kawase et al. [12]. Multiple comparisons without support from functional data can detect relationships that are statistically significant, but may not be clinically or biologically relevant [13]. In vitro assays may help to clarify the situation for certain HLA disparities [34], but this approach is not practical for studying the large number of combinations of HLA mismatches that are observed in clinical transplantation.

This study shows that extraordinary diversity of HLA creates complexity related to multiple HLA disparities between donor and recipient along with differences in the context in which each amino acid difference occurs within HLA molecules. Decades of research in transplant immunology have taught us that these are important factors influencing the ability to stimulate a clinically significant response against an alloantigen. Unfortunately, the relatively large study population examined here ($n = 4226$) provides only a few examples for investigation, and none of these have sufficient subjects to apply robust multivariate statistical approaches. Given extensive HLA polymorphism and complex biology, it is unlikely that the approaches described above will be successful in ranking the relative risks of most of the HLA disparities that are encountered in clinical practice in the United States. Although it may be possible to study certain HLA disparities in specific populations such as the Japanese, these observations may not extend to other racial and ethnic groups because there may be other genetic differences that influence immune responses.

There have been attempts to identify permissive and detrimental HLA disparities using characteristics of HLA mismatches. Several studies have classified HLA disparities based upon typing resolution: low-resolution (antigen level) and high-resolution (allele level) mismatches. This approach assumes that HLA mismatches detected by serologic HLA typing reagents (ie, low resolution) have different clinical effects than those that are not. The reported studies have been conflicting with the largest detecting no statistically significant difference for a single allele versus a single antigen mismatch [1]. One explanation is that this comparison does not adequately classify HLA mismatches according to their risk. The number and function of amino acid differences in each of these groups is diverse, and those with 3-6

Table 5. Number of Subjects Needed to Achieve 80% Power for Detecting an Association with 100 day Mortality for the 6 Most Frequent HLA-A Disparities in HLA-B, -C, and -DRB1 Matched Pairs*

HLA-A Mismatch	Number of Mismatches in 4,226 Subjects	Number Required for 80% Power	Total Number of Subjects Required to Achieve 80% Power
A*0201:0205	27	72	11,269
A*0301:0302	15	4687	1,320,484
A*0201:0206	15	39	10,988
A*0201:6801	11	43	16,520
A*0101:1101	11	2653	1,019,234
A*0101:0201	9	2014	945,685
A*2402:2403	8	30	15,848

*Using the difference observed between each group and an 8/8 match.

amino acid differences can have either high or low resolution HLA disparities (Figure 1).

Scoring systems that group HLA disparities based upon their similarity have also been explored. One approach that assigned scores to HLA disparities based upon the number, position, and properties of the amino acid differences has not correlated well with clinical outcomes [18,19]. This may, in part, be caused by observations showing that the number of mismatched amino acids may not correlate with T lymphocyte responses [34]. Duquesnoy et al [20] have created an algorithm that has shown some promise in solid organ transplantation, but ranking by this approach has not correlated with outcomes in a NMDP data set.

Although these attempts have not been successful, approaches that combine HLA disparities using similar functional characteristics have the highest likelihood for success in studying populations that have a large number of low frequency HLA disparities. One possibility would be to establish a group in which the amino acid differences are predicted to only alter the peptide binding groove (PBG group) versus all others, or perhaps subsets thereof defined by differences in epitopes for T, B, and NK lymphocytes. In this scenario, many high-resolution mismatches that have only 1 amino acid difference in the peptide binding groove (eg, A*0201:0206) would be classified in the PBG group. Classification of HLA mismatches that have amino acid differences outside the peptide binding groove is more complicated. There is evidence suggesting that there are 6 amino acids that are frequently involved in docking of T cell receptors [35]. If these positions are the same between 2 HLA molecules and there are differences in the peptides bound by the HLA molecule, positive selection of T lymphocytes on a similar HLA surface may increase the frequency of alloreactive T lymphocytes. In this case, A*0201:0206, which several studies suggest may be associated with adverse transplant outcomes, may be detrimental because T cells selected by similar docking residues respond to subtle differences in peptides bound by the HLA molecule. HLA disparities with differences in T cell contact residues may be less problematic [34]. Others such as A*0202:0224 would be classified in another group because some of the amino acid differences are located in loops that are predicted to create epitopes for alloantibodies.

Extreme HLA diversity, genetic variation involving other factors influencing immune responses, and numerous factors influencing transplant outcomes (eg, disease and stage of disease, recipient age) create a formidable challenge for achieving compelling data to develop well accepted guidelines for ranking the HLA mismatches in potential donors. Until this problem can be solved, it is tempting to use available reports with statistically significant comparisons to guide donor selection. The observations reported here illustrate

some of the limitations of published investigations and the need for caution in using the available data to guide donor selection. Ultimately, sophisticated models will likely be required to address this important question.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bbmt.2009.04.015](https://doi.org/10.1016/j.bbmt.2009.04.015).

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